



**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY**  
WASHINGTON, D.C. 20460

OFFICE OF  
PREVENTION, PESTICIDES  
AND TOXIC SUBSTANCES

**Note to Reader**

**Background:** As part of its effort to involve the public in the implementation of the Food Quality Protection Act of 1996 (FQPA), which is designed to ensure that the United States continues to have the safest and most abundant food supply. EPA is undertaking an effort to open public dockets on the organophosphate pesticides. These dockets will make available to all interested parties documents that were developed as part of the U.S. Environmental Protection Agency's process for making reregistration eligibility decisions and tolerance reassessments consistent with FQPA. The dockets include preliminary health assessments and, where available, ecological risk assessments conducted by EPA, rebuttals or corrections to the risk assessments submitted by chemical registrants, and the Agency's response to the registrants' submissions.

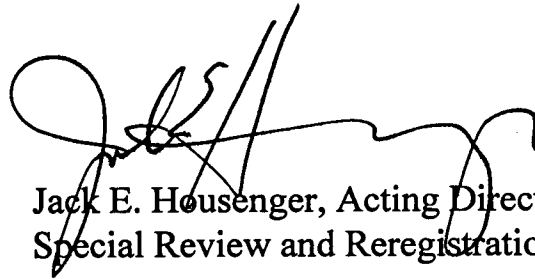
The analyses contained in this docket are preliminary in nature and represent the information available to EPA at the time they were prepared. Additional information may have been submitted to EPA which has not yet been incorporated into these analyses, and registrants or others may be developing relevant information. It's common and appropriate that new information and analyses will be used to revise and refine the evaluations contained in these dockets to make them more comprehensive and realistic. The Agency cautions against premature conclusions based on these preliminary assessments and against any use of information contained in these documents out of their full context. Throughout this process, If unacceptable risks are identified, EPA will act to reduce or eliminate the risks.

There is a 60 day comment period in which the public and all interested parties are invited to submit comments on the information in this docket. Comments should directly relate to this organophosphate and to the information and issues available in the information docket. Once the comment period closes, EPA will review all comments and revise the risk assessments, as necessary.

These preliminary risk assessments represent an early stage in the process by which EPA is evaluating the regulatory requirements applicable to existing pesticides. Through this opportunity for notice and comment, the Agency hopes to advance the openness and scientific soundness underpinning its decisions. This process is designed to assure that America continues to enjoy the safest and most abundant food supply. Through implementation of EPA's tolerance reassessment program under the Food Quality Protection Act, the food supply will become even safer. Leading health experts recommend that all people eat a wide variety of foods, including at least five servings of fruits and vegetables a day.

**Note:** This sheet is provided to help the reader understand how refined and developed the pesticide file is as of the date prepared, what if any changes have occurred recently, and what new information, if any, is expected to be included in the analysis before decisions are made. **It is not meant to be a summary of all current information regarding the chemical.** Rather, the sheet provides some context to better understand the substantive material in the docket ( RED chapters, registrant rebuttals, Agency responses to rebuttals, etc.) for this pesticide.

Further, in some cases, differences may be noted between the RED chapters and the Agency's comprehensive reports on the hazard identification information and safety factors for all organophosphates. In these cases, information in the comprehensive reports is the most current and will, barring the submission of more data that the Agency finds useful, be used in the risk assessments.

A handwritten signature in black ink, appearing to read 'J. Housenger', is written over the typed name and title.

Jack E. Housenger, Acting Director  
Special Review and Reregistration Division

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HED DOC. NO. 013745

DATE: September 21, 1999

MEMORANDUM

**SUBJECT:** *DIAZINON* - REPLACEMENT OF HUMAN STUDY USED IN RISK ASSESSMENTS - Report of the Hazard Identification Assessment Review Committee.

**FROM:** Jess Rowland, Co-Chair  
and  
Pauline Wagner, Co-Chair  
Hazard Identification Assessment Review Committee  
Health Effects Division (7509C)

**TO:** Steve Knizner, Branch Senior Scientist  
Reregistration Branch III  
Health Effects Division (7509C)

**PC Code: 057801**

On February 16, 1999 and again on March 4, 1999, the Health Effect Division's (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the toxicology database for diazinon and selected doses and toxicology endpoints for risk assessment, based solely on **animal toxicity studies**. The HIARC also determined the appropriate uncertainty factors and margins of exposures for dietary and non-dietary risk assessments. **For clarity, transparency, and utility, the decisions made at the previous HIARC meetings along with those made at this meeting are presented in this report. Consequently, the information contained in this report should be used for risk assessments and supersedes all other reports (RfD, TES, HIARC, etc) for diazinon.**

### Committee Members in Attendance

Members present were: David Anderson, William Burnam, Virginia Dobozy, Pam Hurley, Mike Ioannou, Karen Hamernik, Tina Levine, Susan Makris, Nicole Paquette, Kathleen Raffaele, William Sette, Jess Rowland (Co-Chair), PV Shah, Pauline Wagner (Co-Chair).

Other HED staff present at the meeting were: John Doherty, Tim Leighton, David Hrdy, and from Registration Division, Ben Chambliss.

## **I. INTRODUCTION**

In December 10-11, 1998, the Science Advisory Board/Scientific Advisory Panel discussed both the ethical concerns and the scientific merit of using humans subjects for testing pesticides. The Agency is currently developing a policy for the use of human studies in risk assessment. In the interim, HED has taken the following course of action.

In January, 1999, the HIARC developed a specific outline of parameters and questions for the re-examination of human studies. Human studies were used in endpoint selection for risk assessment for eight organophosphates, including diazinon. These studies were re-evaluated according to the parameters and questions developed by the Committee. The HIARC then selected doses and endpoints from toxicity studies with animals for each of these eight organophosphates. The HIARC examined the human data in conjunction with the animal data to determine the appropriate inter-species uncertainty factor.

In the evaluation of the comparative toxicology data in laboratory animals and humans, the Committee relied mainly on the LOAEL for cholinesterase inhibition at comparable time points (duration). The comparative data was evaluated as follows:

If the comparative data indicate (by the dose level and the magnitude of the effect) that humans are more sensitive than laboratory animals, there is no justification for reducing the inter-species uncertainty factor (default value of 10).

If the comparative data indicate (by the dose level and the magnitude of the effect) that humans and laboratory animals are equally sensitive or that humans are less sensitive than laboratory animals, there is a possibility that the inter-species uncertainty factor could be reduced.

On January 14, 1999, using the parameters developed for evaluation of the human studies, the HIARC evaluated the the study conducted in humans subjects with diazinon (Lazanas, 1966). The HIARC classified this study as *unacceptable* because an audit carried out in 1980 (Clements report) classified it as “INVALID” based on the following findings: 1) no physician oversight; 2) no rationale for the ‘normalization’ factor used in data reporting; 3) no analysis of capsules or record of specific dose administered; and 4) no raw data available.

On February 16, 1999 and again on March 4, 1999, the HIARC reevaluated the doses and toxicology endpoints selected for diazinon in order to base all endpoints solely on animal toxicity studies. In addition, the HIARC determined the uncertainty factors and margins of exposures (MOEs), for chronic dietary and short, intermediate and long term occupational exposure risk assessments, respectively.

## **II. HAZARD IDENTIFICATION**

### **A. Acute Dietary Reference Dose (RfD)**

Studies selected:       Acute Neurotoxicity - Rat  
                              Special study to determine the NOAEL for Cholinesterase  
                              Inhibition -Rat

MRID No(s): 43132204 and 44219301

Summary: For acute dietary risk assessment, the HIARC selected the NOAEL of 0.25 mg/kg based on inhibition of plasma cholinesterase activity in female rat observed at 2.5 mg/kg (LOAEL). This NOAEL was established based on the results of two single dose studies in male and female Sprague-Dawley rats.

In the acute neurotoxicity study (MRID No. 43132204), rats (15/sex/dose) received diazinon (D-Z-N technical 88% purity) in corn oil by gavage. at 0, 2.5, 150, 300 or 600 mg/kg. . Clinical signs, FOB and motor activity assessed in 10/se/dose; the other five were assessed for ChE/AChE activity. Plasma cholinesterase activity (ChE) was inhibited at all dose levels (27% for males and 47% for females in the 2.5 mg/kg dose group) and RBC AChE was inhibited at 150 mg/kg (83% for males and 76% for females) at the time of peak effect (about 9 hours postdosing). ChE was equivalent to the controls at day 15 but RBC AChE still remained inhibited for both males and females especially at the higher dose levels. Brain AChE was unaffected when assessed at day 15. The LOAEL for RBC AChE inhibition was 150 mg/kg. The NOAEL for RBC AChE inhibition was 2.5 mg/kg. The LOAEL for plasma ChE inhibition is < 2.5 mg/kg.

Since a NOAEL for plasma cholinesterase inhibition was not established, another study was conducted.

In that study (MRID No. 44219301), at 0, 0.5, 0.5, 1, 10, 100 or 500 to males and at 0, 0.05, 0.12, 0.25, 2.5, 25 or 250 mg/kg to females and sacrificed ~24 hours later. Observations on their behavior reactions were noted and the blood and brain were assessed for ChE/AChE. The precision of the ChE/AChE assays was considered fair to poor but not sufficiently poor to preclude an assessment of the potential for diazinon to inhibit ChE/AChE. Plasma ChE was inhibited at 2.5 mg/kg in females (61%) and at 10 mg/kg in males (44%). RBC AChE was inhibited at 25 mg/kg in females (35%) and at 100 mg/kg in males (49%). Brain AChE was inhibited at 25 mg/kg in females (36%, not significant) and at 250 mg/kg (70%) and at 500 mg/kg in males (69%). **The LOAEL was 2.5 mg/kg based on 61% plasma ChE inhibition in females. The NOAEL is 0.25 mg/kg.**

Dose and Endpoint Selected for Establishing the Acute RfD = 0.25 mg/kg. based on inhibition of plasma cholinesterase activity in female rat observed at 2.5 mg/kg (LOAEL).

Uncertainty Factor (UF): 100 (10 x for inter-species extrapolation and 10 x for intra-species variability).

$$\text{Acute RfD} = \frac{0.25 \text{ mg/kg(NOAEI)}}{100 \text{ (UF)}} = 0.0025 \text{ mg/kg}$$

Comments about Study/Endpoint/Uncertainty Factor: **No change from the previous dose/endpoint based on the rat studies (i.e., human data was not used previously).** The TES Committee did not use the human study because reproducible animal studies were available following a single exposure as demonstrated in the two studies discussed above.

## **B. Chronic Dietary RfD**

Studies selected: 4 week, 90 day and 1 year feeding studies in dogs and 28 day feeding special cholinesterase study, 90 day neurotoxicity, 90 day feeding and chronic feeding studies in rats.

MRID No(s): See Section VII

Dose and Endpoint for establishing the Chronic RfD = 0.02 mg/kg/day based on the consistent pattern of **no** adverse effects on cholinesterase inhibition.

Uncertainty factor = 100 (10 x for inter-species extrapolation and 10 x for intra-species variability).

$$\text{Chronic RfD} = \frac{0.02 \text{ mg/kg/day}}{100 \text{ (UF)}} = 0.0002 \text{ mg/kg/day}$$

Comments about Study/Endpoint/Uncertainty Factor: **This dose and endpoint replaces the previous dose/endpoint based on the human study.**

The endpoint and dose level selected for use in the chronic dietary risk assessment is based upon an analysis of seven oral repeated dose studies, four in the rat and three in the dog. A weight-of-evidence discussion is presented in Section VII of this report.

The results of these studies, taken *in toto*, demonstrated that at 0.02 mg/kg/day a consistent pattern of no adverse effects was achieved. The data for the rat which were considered included: an oral 28-day study, a 90-day feeding study, a 90-day oral neurotoxicity study and a two year feeding study. In the first three studies 0.02 mg/kg was clearly established as a NOAEL based upon statistically significant plasma cholinesterase inhibition at the next higher doses. In the two year feeding study, the dose levels did not include a 0.02mg/kg level, but the lowest two doses, 0.004/0.005 mg/kg in males and females, respectively and 0.06/0.07 in males and females, respectively, bracketed this level. Although at the 0.06 mg/kg level there was statistically significant depression in plasma cholinesterase in females in 4/5 time point measurements, the males (0.07 mg/kg) showed much more variability at this dose and had statistically significant plasma cholinesterase depression only in 1/5 time point measurements. At the lowest dose, 0.004 mg/kg the males exhibited the same variability in plasma cholinesterase measurement although none of the levels reached statistical significance. Given the fact that there is no consistent pattern

of plasma cholinesterase between the sexes, and the 0.06 mg/kg level appears to be a minimal effect level while the 0.004 mg/kg level is clearly a no-effect level, the 0.02 mg/kg level, common to the other three studies, was judged to be an overall NOAEL level for the rat.

The data for the dog which were considered included: a 4-week pilot feeding study, a 90-day feeding study and a one-year feeding study. Each of these studies had a common dose level of 0.02 mg/kg. In each of these studies the only effect seen at that dose level was plasma cholinesterase inhibition. In the 4-week pilot only females had a statistically significant inhibition of plasma cholinesterase which appeared to reach steady state between 14-25 days of dosing. In the 90-day study only males had a statistically significant inhibition of plasma cholinesterase at 0.02 mg/kg and only on days 29 and 86. In this study steady state levels of plasma cholinesterase inhibition were reached between days 30 and 90. In the one year study there were statistically significant decreases in plasma cholinesterase in females in 2/4 time point measurements at the lowest dose of 0.0037, but these decreases were considered not biologically relevant due to the inconsistency across time and the variability of the magnitude of the decreases. At the next dose, 0.02 mg/kg, the only effect observed was statistically significant plasma cholinesterase inhibition in females across all time points and in males only midway in the study at day 176. No other effects were seen in any of the studies at the 0.02 mg/kg dose. The plasma cholinesterase inhibition at 0.02mg/kg is considered to be a minimal or borderline effect in the dog since there were no effects on either the blood or brain cholinesterase levels, and there was no consistent pattern of cholinesterase inhibition between the sexes at this level.

In summary, using a weight of evidence approach, the chronic dietary endpoint is based upon the results of seven studies in the dog and rat which point to 0.02 mg/kg/day as the appropriate level on which to conduct the chronic dietary risk assessment.

Although 0.02 mg/kg/day was selected based on the results of short and long-term studies, no additional uncertainty factors were deemed necessary since: 1) the principal effect (plasma cholinesterase inhibition) was considered to be minimal or borderline, primarily there were no other effects observed at this dose (e.g., no red blood cell or brain cholinesterase inhibition nor clinical signs of toxicity or systemic effects), and there were no consistent pattern of cholinesterase inhibition between the sexes at this level ; 2) a steady state of plasma cholinesterase inhibition was reached by 30 to 90 days in the dog; and 3) this dose (0.02 mg/kg/day) was a clear NOAEL in rats.

## **C. Occupational / Residential Exposure**

### **1. Dermal Absorption**

Dermal Absorption Factor: 100%

A dermal absorption study is not available. The HIARC determined that the 100% default value (equivalent to oral absorption) is appropriate based on the similarity of results observed following oral and dermal administration. Mortality was observed at the same dose (100 mg/kg/day) via the oral and dermal routes in the same species (rabbits). Following oral administration in the developmental study (MRID No. 00079017), 9 of 22

dams died at 100 mg/kg/day and in the 21-day dermal study (MRID No. 40660807), 4/5 males died at 100 mg/kg/day.

The LD<sub>50</sub> studies were compared due to the lack of a common toxicological endpoint in the oral and dermal studies. No cholinesterase activity was measured in the oral developmental studies in rat or rabbits and there is no dermal toxicity study in rats available in the database.

## **2. Short-Term Dermal**

Studies Selected"      Acute Neurotoxicity - Rat  
Special study to determine the NOAEL for Cholinesterase  
Inhibition -Rat

MRID No(s): 43132204 and 44219301

Summary: See Acute Dietary

Dose and Endpoint Selected for Risk Assessment = 0.25 mg/kg based on inhibition of plasma cholinesterase activity in female rat observed at 2.5 mg/kg.

Comments about Study/Endpoint: The HIARC did not use the 21-day dermal toxicity study in rabbits, since the cholinesterase inhibition pattern is different in this species from other species (rats and dogs).

When the cholinesterase inhibition data from the 3 week dermal toxicity study in rabbits was compared with either the oral toxicity studies with similar duration in rats (6-week; MRID No. 41886301) and dogs (4-week; MRID No. 40815004), rabbits appear to be less sensitive than either rats or dogs. Female rabbits were less sensitive for RBC cholinesterase inhibition than either rat or dogs; for plasma cholinesterase inhibition, male rabbit was less sensitive than the male rat or dog; for brain cholinesterase inhibition, the rabbit was of equivalent toxicity to the rat and was less sensitive than dog. In general, for either sex, the rabbit was less sensitive than either the rat or the dog.

The lesser sensitivity observed via the dermal route in rabbits is supported by the fact that the rabbit has a number of unique physiological and biochemical characteristics which can lead to a potential underestimation of the dermal toxicity of a chemical. This is particularly true of organophosphates which require biological activation to the oxon. In humans, activation of S=organophosphates take place in the liver upon the exchange of oxygen for the sulfur atom. This process, however, does not occur to the same extent in the rabbit due to the high levels of arylesterase present in the rabbit blood stream. Arylesterase can rapidly detoxify organophosphates before they reach the liver and are activated. As a result, basing the dermal toxicity study of an organophosphate solely on rabbit dermal toxicity studies may underestimate the toxicity.

Since a dose from an oral study was selected, a dermal absorption rate of 100% should be used for these risk assessments.

### 3. Intermediate-Term Dermal

Studies selected: 90 day and 1 year feeding study in dogs

MRID No(s): 40815004 and 41920001

Dose selected for Risk Assessment = 0.02 mg/kg/day based on the consistent pattern of no adverse effects on cholinesterase inhibition.

Comments about Study/Endpoint: In the **90-day dog feeding study**, no effects were observed in either sex at the lowest dose of 0.0034 mg/kg/day in males and 0.0037 mg/kg/day in females. At the next higher dose of 0.02 mg/kg/day (both sexes), the only effect noted was plasma ChE inhibition reaching statistical significance in males only on days 29 and 86. However, the magnitude of inhibition in males was consistent across time on the days measurements were taken during the study [day 29 (29%), day 56 (27%), day 86 (30%)]. Corresponding values for females (expressed as percent inhibition) ranged from (15 to 17% and were not statistically significant). Examination of the pattern of plasma ChE activity over time indicated that a steady state level of inhibition was reached by 90 days and possibly as early as 30 days (in other words, no considerable increase in plasma cholinesterase inhibition would be expected after 30 to 90 days of continuous dosing). This observation was supported by a similar examination of the blood cholinesterase data in the 1 year study (which also contained a measurement time point at approximately 90 days).

The rationale for not using the 21-day dermal toxicity study in rabbits is provided above under Short-Term dermal.

Since a dose from an oral study was selected, a dermal absorption rate of 100% should be used for these risk assessments.

### 4. Long-Term Dermal

Studies selected: 4 week, 90 day and 1 year studies in dogs and 28 day feeding special cholinesterase study, 90 day feeding, 90-day neurotoxicity and chronic feeding studies in rats.

MRID No(s): See Section VI.

Dose selected for Risk Assessment = 0.02 mg/kg/day based on the consistent pattern of no adverse effects on cholinesterase inhibition.

Comments about Study/Endpoint: This dose was also used for establishing the chronic RfD. This dose was selected based on the weight-of-evidence of the toxicity data ( See Section VII).

Since a dose from an oral study was selected, a dermal absorption rate of 100% should be used for these risk assessments.

## **5. Inhalation (Any Time Period)**

Study selected: 21-day Inhalation Toxicity - Rat

MRID No. 40815002

Executive Summary: In a 21-day inhalation study, four groups of 15/sex Sprague-Dawley strain rats were dosed as control, 0.1, 1, 10 and 100 ug/L of diazinon MG-8 (87% purity) for six hour/day 7 days/week. No symptoms were reported in response to treatment. The LOEL and NOEL are > 100 ug/L for systemic effects. At 0.1 ug/L, plasma ChE was inhibited in males (30%,  $p < 0.05$ ) and females (56%,  $p < 0.05$ ). Progressively higher levels of inhibition were noted at higher doses. RBC AChE was inhibited in males (18%,  $p < 0.05$ ) at 0.1 ug/L and inhibition was progressively greater at higher doses. In females RBC AChE was statistically inhibited (45%) at 1 ug/L. At 1 ug/L brain AChE was inhibited in both males (13%,  $p < 0.05$ ) and females (15%,  $p < 0.05$ ). The LOEL is < 0.1 ug/L based on plasma ChE in both sexes and RBC AChE in males. The NOEL was < 0.1 ug/L for plasma ChE and RBC AChE in males but was > 0.1 ug/L for RBC AChE in females and brain AChE in both sexes.

Dose selected for Risk Assessment = LOAEL = 0.1 µg/L based on plasma cholinesterase inhibition in both sex and red blood cell cholinesterase inhibition in males. The converted dose = 0.026 mg/kg/day.

$$\frac{0.1 \mu\text{g/L} \times 10.26 \text{ L/hr (RV0)} \times 6 \text{ hrs/day (duration)} \times 1\mu\text{g}/1000 \text{ mg (conversion)}}{0.236 \text{ kg (bw)}}$$

$$= 0.026 \text{ mg/kg/day}$$

Comments about Study/Endpoint: This dose should be used for short, intermediate and long-term risk assessments. Since a NOAEL was not established for cholinesterase inhibition, an additional 3x factor is required (i.e., MOE = 300) for inhalation exposure risk assessments.

## **D. Margins Of Exposure for Occupational/Residential Exposures**

A Margin of Exposure (MOE) of 100 is adequate for dermal (occupational and residential) exposure risk assessments. A MOE of 300 (for the use of a LOAEL) is required for inhalation (occupational and residential) exposure risk assessments.

### E. Recommendation for Aggregate Exposure Risk Assessments

For **acute** aggregate exposure risk assessment, the high end exposure values from food plus water should be compared to the acute RfD.

The **Aggregate Risk Index (ARI)** should be used for **Short, Intermediate and Chronic** aggregate risk assessments due to different MOEs for the dermal (MOE=100) and inhalation (MOE=300) routes.

For both **short- intermediate-and long-term exposures**, the aggregate systemic (oral), dermal and inhalation exposure risk assessments are appropriate due to the common toxicological endpoint (cholinesterase inhibition) seen via the three routes.

$$\text{Aggregate MOE}_{(\text{total})} = \frac{1}{\frac{1}{\text{MOE}_{(\text{oral})}} + \frac{1}{\text{MOE}_{\text{dermal (oral equivalent)}}} + \frac{1}{\text{MOE}_{(\text{inhalation})}}}$$

### III. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In a carcinogenicity toxicity study (MRID 00073372), diazinon (98% purity) was administered to groups of Fischer 344 (50/sex) rats at either 400 or 800 ppm (estimated to be 20 and 40 mg/kg/day) for 103 weeks. The control group consisted of 25/sex untreated rats. No systemic effects were reported. The study itself did not provide a basis for concluding that adequate doses were assessed. The dose levels tested are well established from other studies to be moderately strong inhibitors of plasma ChE, RBC AChE and brain AChE. No evidence of compound related tumors was apparent in this study. The LOAEL for systemic effects is > 40 mg/kg/day. **There was no evidence of carcinogenicity**. The doses tested were judged to be adequate to assess the carcinogenic potential of diazinon based on the known property of diazinon to be a moderate inhibitor of ChE/AChE in several other studies at the dose levels tested.

In a carcinogenicity toxicity study (MRID 00073372) diazinon (98% purity) was administered to 50/sex B63CF1 strain mice in their diets at dose levels of 100 or 200 ppm (estimated to be 14 and 29 mg/kg/day) for 103 weeks. The control group consisted of 25/sex untreated mice. No data on systemic effects were seen. **There was no evidence of carcinogenicity**. The doses tested were judged to be adequate to assess the carcinogenic potential of diazinon.

Based on the lack of evidence of carcinogenicity studies in mice and rats diazinon is classified as a **“not likely human carcinogen”**.

#### IV. FQPA ASSESSMENT

##### 1. Adequacy of Data Base

The data base to assess the *in utero* and postnatal exposures of diazinon included prenatal developmental toxicity studies in rats and rabbits as well as a two-generation reproduction study in rats. In addition, adequate neurotoxicity studies, following single and repeated exposures in rats were available to characterize the neurotoxic potential of diazinon.

##### 2. Neurotoxicity

In an acute neurotoxicity screening study (MRID No.: 43132201 and 43132204), groups of 15/sex rats (Sprague-Dawley) were dosed as control 2.5, 150, 300 or 600 mg/kg of diazinon (D-Z-N technical 88% purity) in corn oil by gavage. 10/sex/group were assigned to the main phase of the study to assess for clinical signs, FOB and motor activity; the other five were assessed for ChE/AChE activity. Plasma ChE was inhibited at all dose levels (27% for males and 47% for females in the .5 mg/kg dose group) and RBC AChE was inhibited at 150 mg/kg (83% for males and 76% for females) at the time of peak effect (about 9 hours postdosing). ChE was equivalent to the controls at day 15 but RBC AChE still remained inhibited for both males and females especially at the higher dose levels. Brain AChE was unaffected when assessed at day 15. **The LOAEL for RBC AChE inhibition is 150 mg/kg. The NOAEL for RBC AChE inhibition is 2.5 mg/kg. The LOAEL for plasma ChE inhibition is < 2.5 mg/kg.** Based on the FOB assessments, effects at 150 mg/kg included abnormal gait (3/10 males, 7/10 females), ataxic gait (3/10 females), decreased body temperature (-2.1%, females), decreased rearing counts (-33% females), stereotypy (3/10 females) and fecal consistency and stained fur (3/10 males). Numerous other FOB parameters were affected at 300 mg/kg and above, of these tremors (6/10 females and 5/10 males at 300 mg/kg) were noted and dehydration (6/10 females) were noted. Refer to DER for additional parameters affected. Motor activity was decreased for males (27%, not significant) and females (46%  $p < 0.01$ ) at 150 mg/kg and above. Body weight gain in males was decreased in the 300 (25%) and 600 (29%) mg/kg dose groups. Deaths (2 males and 1 female) resulted at 600 mg/kg. No histopathological lesions attributed to treatment were indicated. **The LOAEL for neurotoxicity is 150 mg/kg based mainly on ataxic gait and supported by other effects believed to be related to ChE/ACHE inhibition. The NOAEL for neurotoxicity is 2.5 mg/kg.**

In a special study (MRID No.: 43132203) especially designed to establish a NOAEL for ChE/AChE, five groups of 15 Sprague-Dawley rats/sex were dosed as control, 2.5, 150, 300 or 600 mg/kg diazinon MG87% (D\*Z\*N, 88% purity) by gavage in corn oil and were sacrificed in groups of 5/sex after 3, 9 or 24 hours. These intervals were designated as pre-peak, peak and post-peak for effects. The rats were assessed for clinical signs and for plasma ChE, RBC and brain AChE. Clinical signs were first evident in the 300 mg/kg dose group males at 9 hours and at 600 mg/kg at 3 hours. Males were more frequently affected than females. Plasma ChE was inhibited at 2.5 mg/kg by 30% for males and 60% for females after 9 hours and to a lesser extent at the other intervals. 66-91% inhibition

was noted for all other intervals at higher doses. RBC AChE was inhibited 40% ( $p < 0.01$ ) in females dosed with 2.5 mg/kg and 42 to 82% at the higher doses for all other intervals. Four brain regions (cerebellum, cerebral cortex, striatum and hippocampus) and the spinal cord were also assessed. Definite brain AChE inhibition (31 to 68%) was noted at 150 mg/kg in all four regions and the spinal cord. Thus, the LEL for plasma ChE and RBC AChE is  $< 2.5$  mg/kg for both sexes but the NOEL and LEL for brain AChE are 2.5 and 150 mg/kg. **Limited correlation between enzyme inhibition with symptoms was apparent since at 9 hours the symptoms were maximal and inhibition ( $> 77\%$  in brain,  $>74\%$  in RBC and  $>77\%$  in plasma at 600 mg/kg) were reported but the enzymes remained inhibited when the symptoms regressed at 24 hours.**

In another study (MRID No.: 44219301) conducted in two parts, to assess for the cholinesterase NOEL and LOEL and neurotoxicity responses following acute administration. In Part 1, behavioral effects and potential for inhibition of ChE/AChE of Diazinon MG87% was assessed in Sprague-Dawley Crl:CD BR/VA/Plus strain rats. Part 1 (behavioral effects), four groups of 5 rats/sex were dosed with 0, 100, 250 or 500 mg/kg of diazinon (undiluted) and additional groups of females were dosed with 25 or 50 mg/kg and the rats observed for clinical signs for 14 days. At 100 mg/kg, females were noted to have one incident of hypoactivity. At 250 and/or 500 mg/kg, miosis, hypoactivity, fur staining, and/or loss of pain reflex and at 500 mg/kg there was one death. These findings were corroborated by the ChE/AChE part of the study which also demonstrated miosis at 100 mg/kg in a single male rat. **The LOEL is 250 mg/kg based on miosis and hypoactivity. The NOEL is 100 mg/kg but this is considered a threshold dose level.**

In Part 2 (ChE/AChE effects), seven groups of males were dosed as control, 0.05, 0.5, 1, 10, 100 or 500 mg/kg and seven groups of females were dosed as control, 0.05, 0.12, 0.25, 2.5, 25 or 250 mg/kg and sacrificed ~24 hours later. Observations on their behavior reactions were noted and the blood and brain were assessed for ChE/AChE. The precision of the ChE/AChE assays was considered fair to poor but not sufficiently poor to preclude an assessment of the potential for diazinon to inhibit ChE/AChE. Plasma ChE was inhibited at 2.5 mg/kg in females (61%) and at 10 mg/kg in males (44%). RBC AChE was inhibited at 25 mg/kg in females (35%) and at 100 mg/kg in males (49%). Brain AChE was inhibited at 25 mg/kg in females (36%, not significant) and at 250 mg/kg (70%) and at 500 mg/kg in males (69%). **The LOEL is 2.5 mg/kg based on 61% plasma ChE inhibition in females. The NOEL is 0.25 mg/kg.**

In a subchronic neurotoxicity study (MRID No.: 43549302) 5 groups of 15/sex Sprague-Dawley Crl CD<sup>R</sup> BR strain rats were dosed as controls, 0.3, 30, 300 or 3000 ppm corresponding to approximately 0.018, 1.8, 18 and 180 mg/kg/day of D\*Z\*N diazinon MG87% for 90 days with periodic assessments for clinical signs and FOB, motor activity and blood ChE/AChE. Regional brain AChE activity and neurohistopathology were assessed at termination. Principal clinical signs included (muscle fasciculations, 8/15 females; hyper-responsiveness and tremors, decrease in grip strength: 15-20% in males and 14-39% in females); body weight and gain and food consumption decrease in both sexes were noted at 3000 ppm only. **The LOEL for systemic and neurotoxicity**

effects is 3000 ppm (180 mg/kg/day) based on weight gain decrease and signs of nervous system perturbation. NOAEL is 300 ppm (18 mg/kg/day). At 30 ppm, plasma ChE (79%-86% in females, 37%-45% in males) and RBC AChE (53-60% in females and 37%-75% in males) and brain AChE cerebral cortex/hippocampus only (25% in females) were inhibited. Other regional brain AChE sources were inhibited at 300 ppm (55%-75% in females) but only at 3000 ppm in males 62% - 73%). Conclusions regarding inhibition of brain AChE are deferred to an accompanying study (MRID No. 43543901) which was especially designed to assess regional brain AChE inhibition. **The LOAEL for plasma ChE and RBC AChE inhibition is 30 ppm and the NOAEL is 0.3 ppm.**

### 3. Developmental Toxicity

In a prenatal developmental toxicity study, four groups of 27 assumed pregnant rats (Charles River Crl. COBST<sup>TM</sup> CD<sup>TM</sup> (SD)(BR)) were dosed as control, 10, 20 or 100 mg/kg/day on days 6 through 15 of gestation. Diazinon (purity not specified) was suspended in 0.2% carboxymethyl cellulose and the rats were dosed by gavage at a rate of 10 mL/kg/day. The rats were sacrificed on day 20 of gestation. MRID No.: 00153017. At 100 mg/kg/day maternal body weight gain was decreased particularly during the 6-10 day interval (-11±2 gms vs +14±2 gms for the control). After that interval the rats showed recovery but net gain was 25% less for the high dose group at sacrifice. **The maternal toxicity LOAEL is 100 mg/kg/day based on body weight gain decrease. The NOAEL is 20 mg/kg/day.** The mean fetal weight in the 100 mg/kg/day dose group was increased (~6%) and the mean number of live fetuses in this groups was slightly reduced. There were also noted slight increases in pre and postimplantation loss. An increase in rudimentary T-14 ribs that was within historical control range was also noted. **The NOAEL for developmental toxicity is 100 mg/kg/day (HDT); a LOAEL was not established.**

In a developmental toxicity study (MRID No.: 00079017) diazinon (89.2% purity suspended in 0.2% carboxymethyl cellulose) was administered by gavage (1 mL/kg) to four groups of assumed pregnant New Zealand White Rabbits at dose levels of 0 (vehicle control), 7, 25 or 100 mg/kg/day on days 6 to 18 of gestation. At 100 mg/kg/day there were 9 deaths in the group of 22 does (40.9%). Clinical symptoms including tremors and convulsions and body weight gain decreases as well as gastro-intestinal hemorrhages and erosions were noted. **The LOAEL for maternal toxicity is 100 mg/kg/day based on deaths. The NOAEL for maternal toxicity is 25 mg/kg/day.** No compound related effects on the fetuses were evident. **The NOAEL for developmental toxicity is > 100 mg/kg/day; a LOAEL was not established.**

### 4. Reproductive Toxicity

In a multi generation reproduction study (MRID No.: 41158101), four groups of 30/sex Sprague-Dawley strain rats were dosed as control, 10, 100 or 500 ppm of diazinon (equivalent to 0, 0.67, 6.69 or 35.15 mg/kg/day in male, and 0, 0.77, 7.63 or 41.43 mg/kg/day in females) for 10 weeks and mated (1:1) to produce F1 litter pups. The F1

litters were culled and mated to produce the an F2 generation.

In the parental groups, at 100 ppm there was decreased weight gain (5-6% persistent for males in the second parental group and transitory for females.). At 500 ppm there were tremors in females; decreased male and female mating and fertility indices (second parental group) and increased gestation length. Dystocia and death were slightly increased but not definitely associated with treatment. **The LOAEL is 100 ppm (6.69 mg/kg/day) based on decreased parental weight gain. The NOAEL is 10 ppm (0.67 mg/kg/day).** In the pups, at 100 ppm there was mortality and decreased weight gain during lactation. At 500 ppm there were decreases litter size and viable pups. **The LOAEL is 100 ppm (6.69 mg/kg/day) based on pup mortality and decreased weight gain. The NOAEL is 10 ppm (0.67 mg/kg/day).**

#### 5. Determination of Susceptibility

Prenatal developmental toxicity studies in rats and rabbits provided no indication of increased susceptibility of rats or rabbit fetuses to *in utero* exposure to diazinon. There was no indication of increased susceptibility in the fetuses as compared to parental animals in the two generation reproduction study. In the prenatal developmental studies no developmental toxicity was seen at the highest dose tested, and in the two-generation reproduction study, effects in the offspring were observed only at treatment levels which resulted in evidence of parental toxicity.

#### 6. Developmental Neurotoxicity

Based on a weight-of-the-evidence basis determined that a developmental neurotoxicity study is **not required** (RfD Report date 6/17/97).

#### 7. Determination of the FQPA Factor:

The FQPA Safety Factor Committee met on June 15 and 16, 1998 to evaluate the hazard and exposure data for diazinon and recommend application of the FQPA Safety Factor (as required by Food Quality Protection Act of August 3, 1996), to ensure the protection of infants and children from exposure to these pesticides.

The FQPA Safety Factor Committee has determined that the 10x FQPA safety factor can be **removed** based on the following factors (FQPA Safety Committee Report dated August 6, 1998):

- (a) In prenatal developmental toxicity studies following *in utero* exposure in rats and rabbits, there was no evidence of developmental effects being produced in fetuses at lower doses as compared to maternal animals nor was there evidence of an increase in severity of effects at or below maternally toxic doses.
- (b) In the pre/post natal two-generation reproduction study in rats, there was

no evidence of enhanced susceptibility in pup when compared to adults (i.e., effects noted in offspring occurred at maternally toxic doses or higher).

- (c) There was no evidence of abnormalities in the development of the fetal nervous system in the pre/post natal studies.
- (d) There is no concern for positive neurological effects from the available neurotoxicity studies or for histopathology in the central nervous system from the other toxicological studies (e.g., subchronic rat, chronic dog, chronic mouse and rat).
- (e) The toxicology data base is complete and there are no data gaps according to the Subdivision F Guideline requirements.
- (f) Adequate actual data, surrogate data, and/or modeling outputs are available to satisfactorily assess dietary and residential exposure and to provide a screening level drinking water exposure assessment.

## V. ACUTE TOXICITY

### Acute Toxicity of Diazinon

Guideline No.	Study Type	MRID #(S).	Results	Toxicity Category
81-1	Acute Oral	41334607	LD <sub>50</sub> = 882 (587-1326) mg/kg ♂ = 968 (731-1283) mg/kg ♀ =936 (742-1180) mg/kg combined	III
81-2	Acute Dermal	41334608	LD <sub>50</sub> ~ 2000 mg/kg ♂ (2/5 died) LD <sub>50</sub> > 2000 mg/kg ♀	II
81-3	Acute Inhalation	41334609	4 hours exposure LC <sub>50</sub> = 6.67 (0.189-242) mg/L ♂ not determined for ♀ = 9.36 (0.35-347) mg/L combined	III
81-4	Primary Eye Irritation	41334610	No corneal involvement. transient conjunctivae irritation (1 hr).	IV
81-5	Primary Skin Irritation	41334611	PIS = 0	IV
81-6	Dermal Sensitization	41334612 232008*	Not a sensitizer in guinea pig. Human study indicates 5-6/56 showed positive sensitization	
81-7	Delayed type neurotoxicity in hens	44132701	No evidence of delayed type neurotoxicity at 100 mg/kg.	

\*Accession No.: MRID No.: not available.

## VI. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE	ENDPOINT	STUDY
Acute Dietary	NOAEL=0.25 mg/kg	Plasma cholinesterase inhibition	Acute Neurotoxicity - Rat Special Study-Rat
	UF =100	<b>Acute RfD = 0.0025 mg/kg/day</b>	
Chronic Dietary	0.02 mg/kg/day	Consistent pattern of no adverse effects on cholinesterase inhibition.	4 week, 90 day and 1-year studies in dog 4 wee, 90 day and 2 -year studies in rat
	UF= 100	<b>Chronic RfD = 0.0002 mg/kg/day</b>	
Short-Term (Dermal) <sup>a</sup>	Oral NOAEL= 0.25 mg/kg/day	Plasma cholinesterase inhibition.	Acute Neurotoxicity - Rat Special study -Rat
Intermediate-Term (Dermal) <sup>a</sup>	0.02 mg/kg/day	Plasma cholinesterase inhibition	90 day and 1-year Studies in dogs
Long-Term (Dermal) <sup>a</sup>	0.02 mg/kg/day	Consistent pattern of no adverse effects on cholinesterase inhibition.	4 week, 90 day and 1-year studies in dog 4 wee, 90 day and 2 - year studies in rat
Inhalation (Any Time Period)	LOAEL=0.1 µg/L	Plasma cholinesterase inhibition	21-Day Inhalation - Rat

a = Since oral values were selected, 100% dermal absorption factor should be used for route-to-route extrapolation.

## VII. WEIGHT OF EVIDENCE APPROACH FOR DETERMINATION OF THE THRESHOLD DOSE FOR CHRONIC RISK ASSESSMENTS

The weight-of-evidence (WOE) approach in the dose and endpoint selection is described below:

1. The first step in the WOE process was to identify the critical species and study/studies for this endpoint. Chronic oral toxicity studies were available for the dog, rat, mouse, and monkey. Less confidence was placed in the 104-week chronic study in monkeys relative to the dog or rat studies due to the lack of dosing solution and purity analysis information, questions about the reliability of cholinesterase activity measurements (particularly in the brain) due to animal moribundity, lack of tabulation of clinical signs, and in general, low numbers of animals on which to base conclusions (MRID 00057664). The 103 week carcinogenicity study in mice was not selected because the critical endpoint, cholinesterase inhibition, was not measured (MRID 00073372).

The dog and the rat were identified by the committee as critical species since these studies (1 year and 90-day dog feeding studies and 2 year rat feeding study) demonstrated the lowest dose levels at which no effects of any type were observed, with the next higher dose levels in these studies being dose levels at which some type of effect was noted.

2. In the next step of the WOE process, all of the available **dog studies** (1-year feeding, 90-day feeding and 4 week feeding pilot) were examined closely with respect to dose, effect, and consistency. The findings were as follows:

**In the 1-year dog feeding study** (MRID 41920001), there were four dose levels of diazinon administered: Low: 0.0032/0.0037, Mid 1: 0.015/0.020, Mid 2: 4.7/4.5, and High: 7.7/9.1 mg/kg/day for males and females, respectively. At the lowest dose administered in females (0.0037 mg/kg/day), a *statistically significant* decrease in plasma cholinesterase activity was seen at two (study days 85 and 268) out of the four (study days 85, 176, 268, and 359) measurement timepoints made during the study. However, this observation was not attributed to treatment nor was it considered to be *biologically relevant* since it occurred at just two measurement timepoints and the magnitude of the decreases were inconsistent across time [day 85 (18%), day 176 (9%), day 268 (28%) and day 359 (9%)]. The magnitude of the decreases fluctuated widely from 9% to 28%. Additionally, there were no other findings at this dose level in either sex. At the next higher dose of 0.015 mg/kg/day in males and 0.020 mg/kg/day in females, the only effect observed was statistically significant plasma cholinesterase (ChE) inhibition of no greater than 24% in males and no greater than 40% in females at any of the measurement timepoints. Decreases in plasma cholinesterase activity in females were generally more consistent across time in magnitude [day 85 (32%), day 176 (40%), day 268 (33%) and day 359 (19%)] and in reaching statistical significance (days 85, 176, and 268) than were the decreases noted at the lowest dose and so were considered to be treatment-related. Somewhat less consistency was noted in data for males at 0.015 mg/kg/day wherein statistically significant plasma ChE inhibition (24%) was found only midway in the study on day 176. Although the degree of inhibition was as low as 5% on day 268, it otherwise ranged from 22 to 24% on three out of four measurement timepoints (days 85, 176, and 359). No cholinesterase inhibition (red blood cell or brain), clinical signs nor systemic effects were observed in either sex at this dose.

Systemic effects (mainly effects on body weight gain, food consumption and serum amylase in one

or both sexes) were not reported until the next higher dose in the study of 4.7 mg/kg/day in males and 4.5 mg/kg/day in females and were accompanied by consistent and statistically significant inhibition in both sexes of plasma ChE of 74% to 86%, red blood cell ChE of 25% to 33%, and brain ChE of 15% to 26% (measured only on day 359 and statistically significant in females only) activities. Similar types of effects were noted at the highest dose administered of 7.7 mg/kg/day in males and 9.1 mg/kg/day in females.

**In the 90-day dog feeding study** (MRID No. 40815004), four doses were administered: Low: 0.0034/0.0037, Mid 1: 0.020/0.021, Mid 2: 5.9/5.6, and High: 10.9/11.6 mg/kg/day for males and females, respectively. No treatment-related effects were observed in either sex at the lowest dose of 0.0034 mg/kg/day in males and 0.0037 mg/kg/day in females. At the next higher dose of 0.02 mg/kg/day (both sexes), the only effect noted was plasma ChE inhibition reaching statistical significance in males only on days 29 and 86. However, the magnitude of inhibition in males was consistent across time on the days measurements were taken during the study [day 29 (29%), day 56 (27%), day 86 (30%)]. Corresponding values for females (expressed as percent inhibition) ranged from (15 to 17% and were not statistically significant). Examination of the pattern of plasma ChE activity over time indicated that a steady state level of inhibition was reached by 90 days and possibly as early as 30 days (in other words, no considerable increase in plasma cholinesterase inhibition would be expected after 30 to 90 days of continuous dosing). This observation was supported by a similar examination of the blood cholinesterase data in the 1 year study (which also contained a measurement timepoint at approximately 90 days).

As in the one year dog study, systemic effects did not occur until the next higher dose of 5.9 mg/kg/day in males and 5.6 mg/kg/day in females and were accompanied by statistically significant inhibition in both sexes of plasma ChE (77 to 81%), of red blood cell ChE (25 to 31%), and of brain ChE (30 to 31%) activities. Similar types of findings were reported at the highest dose of about 11 mg/kg/day. The overall outcome of the two studies, which used generally comparable doses, were similar.

**In the 4-week pilot dog feeding study** (MRID No. 40815004), four doses were administered: Low: 0.02/0.023, Mid 1: 0.073/0.082, Mid 2: 0.80/0.75, and High: 14.7/16 mg/kg/day for males and females, respectively. The results of this pilot study (in which 4 dog/sex/group were used) did not contradict the findings in the other two studies. At a dose common to all of the studies (and the lowest dose tested in the pilot study) of 0.02 mg/kg/day, the only effect noted was statistically significant inhibition of plasma ChE (of about 30%), this time in females only. Looking at the study as a whole, plasma ChE inhibition leveled out by 14 to 25 days of dosing.

3. In the next step of the WOE approach, the dose-effect information from all three dog studies was taken together indicate:
  - ▶ there was inconsistency among studies with regard to the sex in which plasma cholinesterase inhibition reached sustained statistical significance over time at 0.02 mg/kg/day, which suggest a threshold or borderline effect dose in the dog;
  - ▶ in the absence of other effects, the level of statistically significant inhibition (no higher than 30 % in males and 40% in females in any of the studies) occurring at 0.02 mg/kg/day was

considered to be a minimal effect.

- ▶ at higher doses (some 200 fold greater), the systemic effects noted in the studies did not include significant manifestations of cholinergic toxicity (even in the presence of a high magnitude of plasma cholinesterase inhibition (i.e. 70 to 80%) and the presence of red blood cell and brain ChE inhibition);
- ▶ noting the consistency in the pattern of effects observed among the three dog studies, it was judged (considered) to be appropriate to use the plasma cholinesterase data from shorter term studies in conjunction with these data from the chronic study in setting the endpoint;
- ▶ a steady state of plasma cholinesterase inhibition is reached by 30 to 90 days and perhaps earlier.

Therefore, the lowest dose tested in any of the studies (0.0032 mg/kg/day) was not selected because plasma cholinesterase inhibition was the only effect noted at 0.02 mg/kg/day, the next higher dose used in any of the studies. As a result, 0.02 mg/kg/day was selected as the overall critical dose and was considered to be a threshold dose in dogs.

4. In the next step of the WOE process, data from the **rat studies** (2-year feeding, 28-day feeding, 90-day neurotoxicity and 90-day feeding) which is the other critical species, supported the endpoint decision made using the dog studies. The findings were as follows:

In the **two year rat feeding study** (MRID No. 41942002), four doses were administered: Low: 0.004/0.005, Mid 1: 0.06/0.07, Mid 2: 5/6, and High: 10/12 mg/kg/day for males and females, respectively. No treatment-related effects were seen at the lowest dose tested. At the next higher dose in the study of 0.06 mg/kg/day in males and 0.07 mg/kg/day in females, considered to be the LOAEL, the only effect observed was statistically significantly decreased plasma cholinesterase activity. Plasma cholinesterase depression over time for females was fairly consistent in magnitude and in reaching statistical significance at the various timepoints measured during the study [day 88 (58%), day 181 (54%), day 356 (51%), day 552 (45%), day 684 (30%)]. Inhibition was statistically significant on all days but day 684. Plasma cholinesterase depression was much more variable over time with respect to magnitude of inhibition and statistical significance for males at this dose [day 88 (28%), day 181 (12%), day 356 (14%), day 552 (19%), day 684 (51%) with statistical significance being reached only on days 88 and 684]. However, quite a bit of fluctuation (variability) in plasma cholinesterase activity values was also observed over time in males at the next lower dose of 0.004 mg/kg/day where cholinesterase activity was decreased below controls by as little as 1% to 7% on days 552 and 88, respectively, and by as much as 29%, 35% and 42 % on days 181, 356, and 684, respectively, yet statistical significance was never attained. Statistically significant red blood cell and brain cholinesterase inhibition and substantial plasma cholinesterase inhibition were observed in both sexes at the next higher doses (0.06, 5 or 10 mg/kg/day in males and 0.07, 6 or 12 mg/kg/day in females), in the absence of clinical signs or systemic toxicity.

The results of the **28-day** (MRID No. 43543901), **90-day feeding** (MRID No. 40815003) and the **90-day neurotoxicity** (MRID No. 43543802) studies in the rat showed NOAELs centered around 0.02 mg/kg/day. The NOAEL was 0.017 in the 90-day neurotoxicity study, 0.02 mg/kg/day in the

28-day study<sup>1</sup>, and 0.03 mg/kg/day in the 90 day feeding study. The results of these studies were generally consistent among each other as well as with the results of the two year rat study. At doses up to 0.4 mg/kg/day, only blood cholinesterase inhibition was observed. At higher doses of 1.7 to 23.1 mg/kg/day, cholinesterase inhibition (blood or blood and brain depending on the dose and sex) was noted in the absence of clinical signs or systemic toxicity. Systemic toxicity (including muscle fasciculations, tremors, hyper-responsiveness, decreased grip strength, body weight gain and food consumption) was not observed in any of the four studies until much higher doses (~200 mg/kg/day).

5. In the next step of the WOE approach, the dose-effect information from all four rat studies was taken together indicate:
- ▶ there was generally consistency in the pattern of effects noted in all four rat studies;
  - ▶ a steady state of inhibition of the blood enzymes was achieved or closely approached by 4 weeks to 90 days;
  - ▶ using an argument similar to that used for the dog studies, it was considered appropriate to use data from the shorter term rat studies
  - ▶ results of the short term studies provided support for the NOAEL of 0.02 mg/kg/day in rats rather than the lowest dose 0.004 mg/kg/day tested in the 2-year study.

As a result, 0.02 mg/kg/day was selected as the overall critical dose and was considered to be a NOAEL in rats.

6. The final step in the W.O.E. process is to consider the data from the two critical species (7 studies total). The data taken *in toto* indicate that the use of a threshold dose of 0.02 mg/kg/day is appropriate for hazard identification and risk assessment since:
- ▶ the critical effect at this dose, plasma cholinesterase inhibition in the dog, was considered to be a minimal effect when overall dose-effect relationships for blood and brain cholinesterase inhibition and systemic effects among all of the studies for the rat and the dog were examined;
  - ▶ there were no other effects at this dose in the dog; and
  - ▶ 0.02 mg/kg/day was the clear NOAEL in the rat;
  - ▶ there is high confidence in using this approach since all available data in the diazinon oral toxicity database were used in the toxicology endpoint selection for chronic risk assessments.

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<sup>1</sup> Although statistically significant inhibition (25%) of brain cholinesterase was observed in the 90-day neurotoxicity study at a dose of 1.9 mg/kg/day, a special follow-up 28-day feeding study performed specifically to address the significance of this finding did not confirm it even at a slightly higher dose of 2.4 mg/kg/day nor was any cholinesterase inhibition observed at 2.4 mg/kg/day in any of the five brain regions sampled in that study.